#### PATENT COOPERATION TREATY

## **PCT**

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference M/44162-PCT		FOR FURTHER ACTIO	ON	See Form PCT/IPEA/416	
International application No.		International filing date (de	ay/month/year)	Priority date (day/month/year)	
PCT/EP20	04/006564	17.06.2004		18.06.2003	
International Patent	International Patent Classification (IPC) or national classification and IPC				
Applicant BASF AKTIENGESELLSCHAFT					
<ol> <li>This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</li> </ol>					
2. This REP	ORT consists of a total of	8	sheets, including	g this cover sheet.	
3. This repor	rt is also accompanied by A	NNEXES, comprising:			
a. 🛛	(sent to the applicant and	to the International Bureau	) a total of	sheets, as follows:	
	sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).				
sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental					
ь. 🗌	Box. (sent to the International)	Bureau only) a total of (indi	cate type and number	r of electronic carrier(s))	
				, containing a sequence listing and/or tables	
	related thereto, in computer Section 802 of the Administ		licated in the Supple	mental Box Relating to Sequence Listing (see	
4. This repo	rt contains indications relati	ng to the following items:			
В	ox No. I Basis of the	report			
<u></u> В	ox No. II Priority				
🗌 в	Sox No. III Non-establi	shment of opinion with rega	urd to novelty, inventi	ive step and industrial applicability	
	ox No. IV Lack of uni	ty of invention			
В	Box No. V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement			lty, inventive step or industrial applicability;	
	Box No. VI Certain documents cited				
□ В	Sox No. VII Certain defe	ects in the international appl	ication		
В	Box No. VIII Certain observations on the international application				
Date of submission of the demand D		Date	e of completion of thi	is report	
Name and mailing address of the IPEA/EP		Aut	Authorized officer		
Facsimile No.		Telo	ephone No.		

Translation

International application No.
PCT/EP2004/006564

Box	No. I	Basis of the report		
1.		d to the language, this report is based on the international nder this item.	application in the language in w	which it was filed, unless otherwise
		report is based on translations from the original language in his the language of a translation furnished for the purpose		·
1	님	international search (Rule 12.3 and 23.1(b))		
	님	publication of the international application (Rule 12.4)		
	Ш	international preliminary examination (Rule 55.2 and/or		
2.	receiving (	d to the elements of the international application, this rep Office in response to an invitation under Article 14 are re ):	port is based on ( <i>replacement st</i> referred to in this report as "on	neets which have been jurnished to the iginally filed" and are not annexed to
		nternational application as originally filed/furnished		
	the d	lescription:		
	pages	s <u>1-40</u>		as originally filed/furnished
	page		_	
	page	s* r	received by this Authority on _	
	the c	laims:		
	nos.			as originally filed/furnished
	nos.*	*	as amended (together	with any statement) under Article 19
	nos.*	1-21	received by this Authority on	/filed with the demand
ŀ	nos.*	* r	received by this Authority on	
	the d	trawings:		
	sheet	ts		as originally filed/furnished
	sheet	ts* I	received by this Authority on	
	sheet			
		quence listing and/or any related table(s) – see Supplement		
			iai Box Relating to Sequence Li	sung.
3.	L_ The	amendments have resulted in the cancellation of:		
	님	the description, pages		<del></del>
	님	the claims, nos.		
1	님	the drawings, sheets/figs		
	닏	the sequence listing (specify):		
		any table(s) related to sequence listing (specify):		
4.		report has been established as if (some of) the amendme have been considered to go beyond the disclosure as filed		
		the description, pages		
		the claims, nos.		
		the drawings, sheets/figs		
		the sequence listing (specify):		
		any table(s) related to sequence listing (specify):		
*	If item 4 a	applies, some or all of those sheets may be marked "super		

International application No.
PCT/EP2004/006564

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement			
1.	Statement		- 11
	Novelty (	(N) Claims 1-10, 12, 18-21	_ YES
		Claims 11, 13-17	_ NO
4	Inventive	Claims 1-10, 12, 18-21 Claims 11, 13-17	YES NO
	Industria	al applicability (IA) Claims 1-21	_ YES
		Claims	_ NO
2.	Citations an	d explanations (Rule 70.7)	
-	1.	This report makes reference to the following	
	documents:		
	D1:	SILVIA M. GLÜCK ET AL.: "Lactate Racemase as a	
	Versatile Tool for the Racemization of Alpha-		
		Hydroxycarboxylic Acids" CHEMICKE LISTY, Vol. 97,	
		No. 6, 1 June 2003 (2003-06-01), page 363,	
		XP002301107, PRAHA, CZ - according to Prof. Vilim	
		Simanek (co-editor of the Chemicke Listy), this	
	document was made available to the public after		
		the priority date, and is therefore not considered	
ļ		prior art under PCT Rule 64.2	
	D2:	SCHNELL, BARBARA ET AL: "Enzymatic racemization	
		and its application to synthetic	
	biotransformations", ADVANCED SYNTHESIS &		
		CATALYSIS, 345(6+7), pages 653-666, CODEN: ASCAF7;	
		ISSN: 1615-4150, 13 June 2003 (2003-06-13),	
		XP002301108	
	D3:	LIU S-Q: "Practical implications of lactate and	
		pyruvate metabolism by lactic acid bacteria in	
		food and beverage fermentations." INTERNATIONAL	
		JOURNAL OF FOOD MICROBIOLOGY, Vol. 83, No. 2, 15	
		June 2003 (2003-06-15), pages 115-131 ,	

International application No.
PCT/EP2004/006564

	FC1/EF2004/000304		
Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement		
	XP002301109, ISSN: 0168-1605		
D4:	STRAUSS U T ET AL: "Deracemization of (+-)-		
	mandelic acid using a lipase-mandelate racemase		
	two-enzyme system" TETRAHEDRON ASYMMETRY 1999,		
	UNITED KINGDOM, Vol. 10, No. 21 , 1999, pages		
	4079-4081, XP002301110, ISSN: 0957-4166		
D5:	FELFER ULFRIED ET AL: "Substrate spectrum of		
	mandelate racemase. Part 2. (Hetero)-aryl-		
	substituted mandelate derivatives and modulation		
	of activity", JOURNAL OF MOLECULAR CATALYSIS B		
	ENZYMATIC, Vol. 15, No. 4-6, 1 November 2001		
	(2001-11-01), pages 213-222, XP002301111, ISSN:		
	1381-1177		
D6:	SCHAFER SUSAN L ET AL: "Mechanism of the reaction		
	catalyzed by mandelate racemase: Structure and		
	mechanistic properties of the D270N mutant",		
	BIOCHEMISTRY, Vol. 35, No. 18, 1996, pages 5662-		
	5669, XP002301112, ISSN: 0006-2960		
D7:	GARCIA-VILOCA M ET AL: "A QM/MM study of the		
	racemization of vinylglycolate catalyzed by		
	mandelate racemase enzyme", JOURNAL OF THE		
	AMERICAN CHEMICAL SOCIETY, 31 JAN 2001, Vol. 123,		
	No. 4, 31 January 2001 (2001-01-31), pages 709-		
	721, XP002301113, ISSN: 0002-7863		
D8:	RONGSHI LI ET AL.: "Racemization of Vinylglycolate		
	by Mandelates Racemase", JOURNAL OF ORGANIC		
	CHEMISTRY, Vol. 60, No. 11, 1995, XP002301114		
D9:	EP-A-0 596 466 (TANABE SEIYAKU CO), 11 May 1994		
	(1994-05-11)		

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2. Novelty and inventive step (PCT Article 33(2) and 33(3))
- 2.1 The subject matter of the present application is a process for isomerising alpha-hydroxycarboxylic acids using microbial lactate racemases with a broader activity spectrum, said lactate racemases per se and the nucleic acids that encode the same, screening methods for said racemases, expression vectors, recombinant micro-organisms which express the racemases, and processes for producing and isolating a protein with alpha-hydroxycarboxylic acid racemase activity.
- 2.2 The prior art, as disclosed in documents D2 to D9, describes racemisation processes of alphahydroxycarboxylic acids using enzymes or microorganisms with high substrate specificity. Lactate racemases with a broader substrate spectrum, i.e. enzymes which racemise lactate and alphahydroxycarboxylic acids of general formula (I), and hence also their uses, are novel.

  Consequently, the present claims 1-10, 12 and 18-21 meet the requirements of PCT Article 33(2) for novelty.

The enzymes per se, as well as the screening methods as per claims 8, 11 and 13-17, are not exclusively directed to lactate racemases with a broader substrate spectrum (i.e. with compounds of general formula (I)). The screening method as per claim 8 requires micro-organisms which express

PCT/EP2004/006564

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

alpha-hydroxycarboxylic acid-racemase activity and form or metabolise lactate, the latter activity not being necessarily linked to the racemase activity, i.e. enzymes with a narrow substrate spectrum are also screened. Already known enzymes and methods for isolating the same are thus covered by the claims, although claims 11-13 and 17 are not novel in the case of these subjects (see D6, Materials and Methods). As for the lactate racemases with a broader substrate spectrum, as mentioned in claim 1, they should be considered novel.

Documents D2 to D6 can be regarded as the closest 2.3 prior art. Those documents disclose racemisation processes using lactate or mandelate racemases with the known restrictions with regard to the substrates. Consequently, the present invention can be considered to address the technical problem of finding a biocatalyst suitable for isomerising alpha-hydroxycarboxylic acids, which do not belong to the substrate spectrum of mandelate racemase, which have a broader substrate spectrum in comparison with lactate racemase, and which can be used in corresponding isomerisation processes. The solution is the process as per claim 1, in which a lactate racemase (for example from the Lactobacillus spp. mutants as per claim 6) with a broader substrate spectrum is used. The prior art contains no indication of lactate racemase enzymes including compounds of general formula (I) with a broader substrate specificity, i.e. these subjects

International application No.
PCT/EP2004/006564

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement can be considered to involve an inventive step in relation to the prior art. Since the screening methods and the enzymes per se are limited by the same restrictions, claims 1-10, 12 and 18-21should be recognised to involve an inventive step. Industrial applicability (PCT Article 33(4)) 2.4 All the present claims meet the requirements of PCT Article 33(4) for industrial applicability.

International application No.
PCT/EP2004/006564

Suppl	lemental	Box
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In case the space in any of the preceding boxes is not sufficient. Continuation of:

## BOX I

1. The claims 1-21 filed with the demand for international preliminary examination meet the requirements of PCT Article 34(2) and have therefore been used as the basis for the international preliminary examination.